



Growth test via plating assay.

Introduction

Aim of this test is to show that the modified strains in this MCAN indeed can use either xylose or arabinose as single carbon source for growth.

In this MCAN strains are described that are developed to produce ethanol starting with pentoses as carbon source.

Lignocellulosic residues, composed of cellulose, hemicellulose and lignin are the most abundant renewable resources available for the production of bioethanol. Cellulose is a homopolymer of glucose, while hemicellulose is composed of a mixture of hexoses (glucose, mannose and galactose) and pentoses (xylose and arabinose). These sugar residues might have additional modifications in the form of acetyl or ferulic acid groups. Economic feasibility of cellulosic ethanol at a commercial scale depends on the conversion of all available sugars present in the lignocellulosic biomass.

Saccharomyces cerevisiae, the most commonly used yeast for industrial ethanol production, is unable to effectively ferment the pentose fraction, being the second most abundant sugars in hydrolysates. Increased commercial interest in fermenting renewable resources to ethanol have led to the development of several recombinant, evolved pentose fermenting *Saccharomyces cerevisiae* strains.

The background strains in this MCAN were selected based on either a robust industrial strain capable of hexose fermentation in toxic hydrolysates or a continuation/improvement of the strain described in MCAN (J13 0007).

Material en Methods

Yeast strains were taken from the DSM strain collection.

Yeast strains were grown on solid YPD (1% Yeast Extract, 2% peptone and 2% glucose) or YNB medium (0,67% Yeast Nitrogen Base) with 2% glucose, 2% xylose or 2% arabinose. Medium is solidified by adding 2% agar. All media were sterilized by autoclaving. Sugar solutions were autoclaved separately as 50% stock solutions for glucose and xylose and as 25% stock for arabinose.

YNB Plates were incubated at 32°C for 3 days. YPD plates were incubated at 32°C for 16-24h.

Strains are precultured on YPD plates, with toothpicks a small amount of cells is transferred to the series of YNB test plates supplemented with either arabinose, xylose or glucose.

Results and discussion

Both the parental, non modified stains and the genetically modified strains with xylose and arabinose fermenting characteristics were tested on the different sugar containing growth media.

The result was scored as negative if no colony forming is observed.

In figure 1 a typical result is shown.

Without exception the parental strains only grew on glucose containing medium, the genetically modified strains did grow on all three carbon sources.

Prolonged incubations (data not shown) up to two weeks did no change the outcome of the experiments. The parental strains scored negative on both [REDACTED] and [REDACTED] supplemented medium.

The conclusion of this experiment is that the *S.cerevisiae* strains tested, cannot utilize [REDACTED] without being genetically modified. The use of renewable plant biomass, can only be optimized with *Saccharomyces cerevisiae* strains genetically modified for [REDACTED].

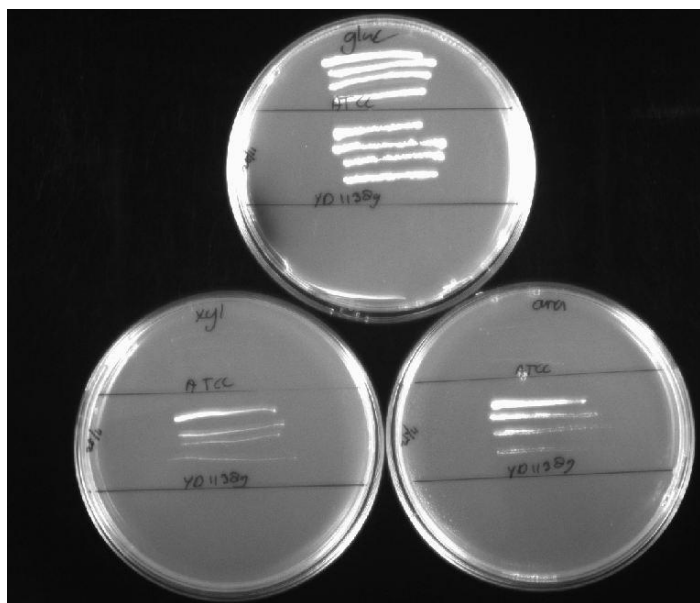


Figure 1 growth on plates supplemented with only one carbon source, top: glucose, bottom left: [REDACTED] and bottom right: [REDACTED]. The Non GMO parental strain is inoculated at the upper part of the plates, the Genetically Engineered [REDACTED] in the middle part. Parental strains failed to grow on the pentose containing plates, the modified stains are capable of growing on these carbon sources.